

*What Is Claimed Is:*

1. An artificial mammalian chromosome comprising essentially centromeric, telomeric, and genomic DNA.

2. An artificial mammalian chromosome comprising essentially centromeric DNA, telomeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

3. An artificial mammalian chromosome comprising essentially centromeric DNA, telomeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

4. An artificial mammalian chromosome produced by the process of transfecting a mammalian cell with purified DNA, said DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

5. An artificial mammalian chromosome produced by the process of transfecting a mammalian cell with purified DNA, said DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E

during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

6. An artificial mammalian chromosome produced by the process of transfecting a mammalian cell with purified naked DNA, said DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

7. An artificial mammalian chromosome produced by the process of transfecting a mammalian cell with purified naked DNA, said DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

8. An artificial mammalian chromosome produced by the process of transfecting a mammalian cell with purified condensed DNA, said DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

9. An artificial mammalian chromosome produced by the process of transfecting a mammalian cell with purified condensed DNA, said DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-

sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

5 10. An artificial mammalian chromosome produced by the process of transfecting purified coated DNA into a mammalian cell, said DNA comprising essentially a centromere, a telomere, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

10 11. An artificial mammalian chromosome produced by the process of transfecting purified coated DNA into a mammalian cell, said DNA comprising essentially a centromere, a telomere, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromere comprises a DNA sequence that associates with CENP-E during mitosis, and said telomere comprises tandem repeats of the sequence TTAGGG.

15 12. The artificial mammalian chromosome of any of claims 4-11, wherein said centromeric DNA, said telomeric DNA and said genomic DNA are not ligated to each other.

20 13. The artificial mammalian chromosome of any of claims 4-11, wherein one or more of said centromeric DNA, said telomeric DNA and said genomic DNA are ligated to one another.

14. A composition comprising the artificial mammalian chromosome of any of claims 1-11.

15. The artificial mammalian chromosome of any of claims 1-11, wherein said centromeric DNA comprises alpha-satellite DNA.

16. A mammalian cell comprising the artificial mammalian chromosome of any of claims 1-11.

5 17. The artificial mammalian chromosome of any of claims 1-11, wherein said chromosome further comprises a heterologous DNA that is expressed from said chromosome, or causes expression of a gene product, when said chromosome is introduced into a mammalian cell.

10 18. Purified DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

15 19. Purified DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-B during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

20 20. Purified naked DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

21. Purified naked DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

22. Purified condensed DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, wherein said DNA is coated with a DNA-condensing agent.

23. Purified condensed DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG, wherein said DNA is combined with a DNA-condensing agent.

24. Purified coated DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, wherein said DNA is coated with one or more DNA-binding proteins.

25. Purified coated DNA comprising essentially telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG, wherein said DNA is coated with one or more DNA-binding proteins.

26. Purified DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

27. Purified DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

28. Purified naked DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

29. Purified naked DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

30. Purified condensed DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, wherein said DNA is combined with a DNA-condensing agent.

31. Purified condensed DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG, wherein said DNA is combined with a DNA-condensing agent.

32. Purified coated DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, wherein said DNA is coated with one or more DNA-binding proteins.

33. Purified coated DNA made by the process of combining, *in vitro*, telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG, wherein said DNA is coated with one or more DNA-binding proteins.

34. A composition comprising the DNA of any of claims 18-33.

35. A mammalian cell comprising the purified DNA of any of claims 18-33.

36. The purified DNA of any of claims 18-33, wherein said centromeric DNA, said telomeric DNA and said genomic DNA are not ligated to each other.

37. The purified DNA of any of claims 18-33, wherein one or more of said centromeric DNA, said telomeric DNA and said genomic DNA are ligated to each other.

38. The purified DNA of any of claims 18-33, wherein said centromeric DNA comprises alpha-satellite DNA.

39. The purified DNA of any of claims 18-33, wherein said DNA further comprises heterologous DNA that is expressed from said chromosome, or causes expression of a gene product, when said DNA is introduced into a mammalian cell.

40. A vector comprising the DNA of any of claims 18-33.



41. A cell comprising the vector of claim 40.

42. A composition comprising the vector of claim 40.

43. A method of making an artificial mammalian chromosome, said method comprising introducing the purified DNA of any of claims 18-33 into a mammalian cell.

44. A method of making an artificial mammalian chromosome, said method comprising introducing the composition of claim 34 into a mammalian cell.

45. A method of making a purified DNA composition, said method comprising combining, *in vitro*, purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

46. A method of making a purified DNA composition, said method comprising combining, *in vitro*, purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

47. A method of making a purified naked DNA composition, said method comprising combining, *in vitro*, purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

48. A method of making a purified naked DNA composition, said method comprising combining, *in vitro*, purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

49. A method of making a purified condensed DNA composition, said method comprising combining, *in vitro*, a DNA-condensing agent and purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

50. A method of making a purified condensed DNA composition, said method comprising combining, *in vitro*, a DNA-condensing agent and purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

51. A method of making a purified coated DNA composition, said method comprising combining, *in vitro*, one or more DNA-binding proteins and purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments.

52. A method of making a purified coated DNA composition, said method comprising combining, *in vitro*, one or more DNA-binding proteins and purified telomeric DNA, centromeric DNA, and genomic DNA, wherein said genomic DNA is a sub-genomic DNA fragment selected from the group consisting of restriction enzyme digestion fragments and mechanically-sheared fragments, said centromeric DNA comprises a DNA sequence that associates with CENP-E during mitosis, and said telomeric DNA comprises tandem repeats of the sequence TTAGGG.

53. The method of any of claims 43-52, wherein said centromeric DNA, said telomeric DNA and said genomic DNA are not ligated to each other.

54. The method of any of claims 43-52, wherein one or more of said centromeric DNA, said telomeric DNA and said genomic DNA are ligated to each other.

55. A method of expressing a gene in a mammalian cell, said method comprising propagating a mammalian cell containing the artificial chromosome of any of claims 1-11, wherein said chromosome contains said gene or contains a DNA sequence that allows expression of said gene.

56. A method of expressing a heterologous gene in a mammalian cell, said method comprising propagating a mammalian cell containing the DNA of any of claims 18-33, wherein said DNA contains said gene or contains a DNA sequence that allows expression of said gene.

5 57. The method of claim 55, wherein said gene expression provides a therapeutic benefit to a mammal comprising said cell.

58. The method of claim 56, wherein said gene expression provides a therapeutic benefit to a mammal comprising said cell.

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